REMARKS

This response is due on January 12, 2004, by virtue of the attached petition and fee for a two-month extension of time. No additional fees are believed to be due; however, should any fees be properly due in connection with the filing of this document, or should the extension of time fee be inadvertently omitted, the Commissioner is hereby authorized to deduct any such fees from Marshall, Gerstein & Borun, LLP account number 13-2855.

In view of the amendments and remarks presented herein, the applicants request withdrawal of the rejections and favorable reconsideration of the claims.

I. Status of the Claims

Claims 1-38 are pending in the instant application. Claims 12-19 were withdrawn as being directed to non-elected species and claims 1-11 and 20-38 stand variously rejected under 35 U.S.C. §112 second paragraph as being indefinite for failing to particularly point out and distinctly claim the invention, and under 35 U.S.C. §103(a). Applicants respectfully traverse the rejections and request reconsideration in light of the above amendments and the following remarks.

Claims 3, 6 and 20 are cancelled herein and claim 1 has been amended to incorporate the limitations of claim 6.

II. Restriction/Election

The Examiner indicated (office action page 2) that claims 12-19 are withdrawn as directed to non-elected species, but that the restriction requirement with respect to Group I claims (*i.e.*, Claims 1-27 and 37-38) and Group II claims (*i.e.*, claims 28-36) is withdrawn. Thus, the claims pending at the time of examination were claims 1-38, however, pursuant to a requirement for an election of species, claims 12-19 were withdrawn as directed to subject matter of non-elected species. Applicants reserve the right to request rejoinder of these withdrawn claims upon an indication of allowance of the remaining claims.

III. Rejection under 35 U.S.C. §112, second paragraph should be withdrawn

Claim 6 was rejected under 35 U.S.C. §112, second paragraph as allegedly being indefinite for failing to point out and distinctly claim the subject matter which Applicants regard as the invention. Cancellation of claim 6 renders the rejection moot.

IV. Rejection under 35 U.S.C. §103(a) should be withdrawn

Claims 1-11 and 20-38 were rejected under 35 U.S.C. §103(a) as allegedly being unpatentable under 35 U.S.C. §103(a) over the teachings of Yao *et al.*, (Toxic. Methods, 2(3)199-218, 1992); Chung *et al.*, (Chonnam J. Med. Sci 1(2) 128-138, 1988) Garza-Ocanas *et al.*, (Toxicol., 73(2)191-201, 1992) or Morrison *et al.* (Biomaterials, 16(13) 987-92, 1995). Each of these references were separately cited as rendering the claimed invention obvious and were not used in combination, as such, the following remarks address the Examiner's characterization of each of these references separately as applied to the claims of the present application. In brief, Applicants respectfully traverse the rejections and request reconsideration of the Application because none of these references disclose the methods of the present invention or provide a suggestion or motivation to arrive at such methods.

a. Yao et al., does not render obvious the claimed methods

The Examiner cited Yao *et al.* as teaching the evaluation of "irritancy potential of chemicals by culturing rabbit conjunctival epithelial cells. Injury to these cells by surfactants . . . assessed by lactate dehydrogenase assay, a neutral red assay and an MTT assay" and stated that the Yao et al. reference provides a correlation between these assays and the Draize test to evidence in vivo toxicity of the surfactants. (See office action page 4).

It is the Examiner's contention that the prior art indicates that it is conventional to: use multiple tests to evaluate the toxicity of a compound; use multiple concentrations in such test; and use controls in such tests. The Examiner then goes on to state that it would have been obvious to "select at least three conventional cytotoxicity tests, to use multiple concentrations, to use a control and to graph the results." (See office action page 4) Applicants respectfully disagree with the Examiner's assessment that the methods of the invention relying on TC_{50} and Ctox would have been obvious. Initially, Applicants note that the claims 1-11, and 21-38 all

refer to at least a calculation of Ctox using NOEL and claim 20 relies on an initial calculation of TC_{50}. Claim 20 was the only claim that relied on TC_{50} alone as a predictor of Ctox. As claim 20 is cancelled herein, any discussion based on calculation of TC_{50} is rendered moot.

Claim 1 and those claims dependent therefrom all recite that Ctox is selected as a concentration that is less than or equal to "the highest concentration of the chemical compound at which a measurable toxic effect . . . is not observable (NOEL)." The methods of claims 1-11 and 21-38 thus rely on an assessment that requires the concentration of the test compound to be limited such that it produces ***no toxic*** effect in multiple assays, and that concentration is calculated from those multiple assays as a discrete concentration at which there is "no observable effect" in any of those assays. This selection has not previously been described in a cytotoxicity cluster assay as described in the present invention.

While the Yao et al. may well teach how to perform three different assays and may well correlate the results of each of those assays ***individually*** with the Draize test for eye irritancy, there is no suggestion anywhere in the art itself (Yao et al. or any other art cited by the Examiner) that teaches that one skilled in the art should determine that concentration of the irritant compound (one of three surfactants in Yao et al.) that has ***no observable effect in all three assays*** and use that concentration as a measure of in vivo toxicity. Indeed from the data presented in Figures 5 (LDH leakage assay), 7 (Neutral Red uptake assay), and 8 (MTT assay) of Yao et al. it is not possible to determine that concentration of the test compound that had no observable toxic effect ***in all*** three assays because, even with the lowest concentration used, the test compounds used by Yao produced some toxic effect in at least Figures 5 and 7. Thus, there is no teaching or suggestion in Yao et al. that one skilled in the art should seek a Ctox value that is equal to or lower than the NOEL found in a cluster of at least three different assays of cytotoxicity.

Thus, the disclosure of Yao *et al.* would not teach one skilled in the art to accumulate the data from each of at least three assays and determine a single threshold concentration at which there is no observable toxic effect and use that as the predictor of the *in vivo* toxicity (Ctox) of a given compound. Without such an express teaching in the cited art the rejection is flawed because it fails to teach an express element of the claimed method (*i.e.*, selecting the Ctox

as described in step (d)(iii)).

The Examiner's statement that "since each test measures the toxicity of the test compound differently, it would have been illogical to pick a concentration that may have little effect on membrane integrity but shows toxicity in mitochondrial function. Determination of an across-the board lack of toxicity of a specific concentration would have been obvious to one of ordinary skill in the art," amounts to hindsight reconstruction of the claimed subject matter by using the application as a roadmap. Such hindsight reconstruction is impermissible because it derives the motivation or suggestion to arrive at the claimed subject matter from the application itself rather than from the art.

The Examiner has not cited, and Applicants see no evidence of a teaching of identifying the NOEL concentration which is the highest concentration of the chemical compound at which a measurable toxic effect of the chemical is not observable (required element of claim 1) in at least three assays; determining a TC_{50} for each of the indicators of cell health (required element of claim 4); a method of identifying a lead compound for drug development (claim 31) or numerous other features of the other dependent claims. Thus, Applicants submit that the Examiner has failed to establish that any of the claims are rendered obvious by Yao *et al.* and Applicants request that the rejection be withdrawn.

b. Chung et al., does not render obvious the claimed methods

The Examiner cites Chung *et al.* and states that it "evaluates cytotoxicity of adriamycin using a neutral red assay, an MTT assay, and LDH assay and a protein content assay. Controls are used for comparison and multiple concentrations of adriamycin are tested." With the exception of these statements, there is no further discussion of Chung *et al.* in the office action.

Chung *et al.* merely was presenting a study of the effects of adriamycin on mycocardial and endothelial cells to arrive at the conclusion that this agent "inhibits in vitro proliferation and growth of the cells by disturbing the cell metabolism" (See abstract). However, at no point throughout the disclosure of Chung *et al.* is it taught or suggested that identifying the NOEL concentrations of adriamycin should be accomplished in three or more assays and then

used as a measure of Ctox to predict the *in vivo* cytotoxicity of adriamycin. In the absence of that teaching, the disclosure of Chung *et al.* is flawed for the same reasons as provided for Yao *et al.* above.

c. Morrison et al., does not render obvious the claimed methods

The Examiner's characterization of Morrison *et al.* was as follows "Morrison et al evaluates the biocompatibility of two polymers that may be used in orthopedic implants by doing a cell protein assay, a GSH assay, a LDH assay and an MTT assay on osteoblasts exposed to the polymers." Again, as with Chung *et al.* and Yao *et al.*, there is nothing in Morrison *et al.* that teaches the use of multiple assays to first identify a NOEL concentration of the compound being tested and then use that NOEL as measure of the in vivo cytotoxicity of the compound as taught by the present invention. In the absence of such a teaching or suggestion in Morrison *et al.* (or some other art that must be cited) and in the absence of any additional indication from the Examiner as to why one skilled in the art would be motivated to determine the NOEL concentration of the polymers discussed by Morrison *et al.*, Applicants maintain that Morrison *et al.* fails to render obvious the claimed methods.

d. Garza-Ocanas et al., does not render obvious the claimed methods

Garza-Ocanas *et al.* is cited by the Examiner as having "assessed and compared the in vitro toxicity of two buckthorn toxins on cultures of hepatocytes and keratinocytes. Cytotoxicity was measured by LDH assay, MTT assay and neutral red assay. Multiple concentrations of toxins were tested." Again, there is no teaching in Garza-Ocanas *et al.* that a NOEL concentration of the test compound should be determined by conducting at least three cytotoxicity assays at varied concentrations to find a Ctox value as taught by the present invention. Indeed even if one were to search for such a value in Garza-Ocanas *et al.*, the data presented in that document would not identify a NOEL value. For example, taking the data of Figure 3 (LDH leakage induced by T544 in hepatocytes) and Figure 5 (effects of T544 on neutral red uptake in hepatocyte), and even if one overlooks the fact that a dose response curve is not provided for the third measure of toxicity (MTT), it would be impossible to determine the NOEL from the presented data because even at the lowest concentration there was an observable effect

on neutral red uptake by the cells (See Figure 5 of Garza-Ocanas *et al*). Furthermore, evidence from Garza-Ocanas *et al.* suggests that any one of the assays (MTT, LDH leakage or NR assay) alone could be used to determine cytotoxicity. For example, at page 266 Garza-Ocanas *et al.* state that "Any of the three endpoints used were able to detect the in vitro hepatotoxicity of the toxins." This teaching alone is sufficient to discourage one skilled in the art to use multiple assays as taught by the present invention.

In view of the above comments the rejections based on Garza-Ocanas *et al.* should be withdrawn.

V. Concluding Remarks

As Applicants note above, while the Examiner cited four references in the rejection of the claims under 35 U.S.C. §103(a), each of those references were cited separately, and not in combination. Thus, the comments provided above address the articulated rejection in its entirety. Applicants believe that each of the presently pending claims in this application is believed to be in condition for allowance. Should the Examiner have any further questions, she is invited to contact the undersigned.

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Respectfully submitted,

By _____

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